INTRODUCTION

Regular physical exercise is highly recommended in the management of type 2 diabetes mellitus (T2DM), and identifying highly effective training strategies is one of many challenges. Training in the overnight-fasted state has received considerable attention and has recently been discussed as a novel and effective training strategy for T2DM patients in a state-of-the-art review on a theoretical basis.

It has been demonstrated that acute exercise in the fasted state can be accompanied by elevated blood free fatty acid concentrations and increased fat oxidation rates in healthy subjects. Furthermore, some studies involving healthy subjects indicate that physical training (regular physical...
exercise) in the fasted state can induce greater adaptations than in the fed state (e.g., increased glucose tolerance, increased basal muscle glycogen concentrations, and up-regulation of components of the oxidative metabolism).8-11 These responses/adaptations could be beneficial in particular in improving the health of (overweight/obese) T2DM patients and affect their glucose regulation and lipid profile. Moreover, it has been shown that both intermittent fasting as well as regular physical exercise can decrease pro-inflammatory molecule levels in healthy subjects.12,13 However, whether and to what extent regular physical exercise in the fasted state can attenuate inflammatory conditions has not yet been examined. To date, the effects in T2DM patients who exhibit chronic low-grade inflammation (that can play a crucial role in the development of insulin resistance)14 are completely unknown.

This is the first study to explore the hypothesis that physical training before breakfast in the overnight-fasted state is more effective in improving the health (body mass index (BMI) and composition, glucose regulation, lipid profile, and (anti-/pro-)inflammatory molecule levels) of T2DM patients than physical training in the fed state.

2 | MATERIAL AND METHODS

The study is registered at the German Clinical Trials Register (registration ID: DRKS00011714). The trial was intended as a pilot study. The protocol for the research project was approved by the local Ethics Committee of the IST University of Applied Sciences Düsseldorf before the investigation. It conformed to the provisions of the Declaration of Helsinki. Written informed consent was obtained from all subjects. The manuscript was prepared in accordance with the CONSORT guidelines for clinical studies.15

2.1 | Study design

The patients (n = 30) were randomly assigned to one of two groups (F group: training in the overnight-fasted state (n = 15) and C group: training in the fed state (control group, n = 15)). Subjects were assigned to the two groups at a ratio of 1:1 through computerized block randomization using the “SPSS” program (v. 24.0, SPSS Incorporation) (Figure 1). All patients completed an 8-week training program. The subjects’ data were analyzed 3-5 days pre-training (T1) and 3-5 days after

FIGURE 1 CONSORT flow diagram
<table>
<thead>
<tr>
<th>Variable</th>
<th>Difference F group vs C group T1</th>
<th>Data from both training groups T1</th>
<th>Data from both training groups T2</th>
<th>F group T1</th>
<th>F group T2</th>
<th>C group T1</th>
<th>C group T2</th>
<th>ANOVA: time effect T1-T2</th>
<th>ANOVA: time × group</th>
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<tbody>
<tr>
<td><strong>Endurance performance</strong></td>
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<tr>
<td>Time to physical exhaustion [s] during WHO step test</td>
<td>$P = .161^{b}$</td>
<td>634 ± 179</td>
<td>700 ± 198</td>
<td>588 ± 162</td>
<td>639 ± 169</td>
<td>680 ± 189</td>
<td>761 ± 211</td>
<td>$P &lt; .001^{a}$</td>
<td>$P = .301$</td>
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<td><strong>Strength performance</strong></td>
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<tr>
<td>5-RM chest presses [kg]</td>
<td>$P = .082^{b}$</td>
<td>33.2 ± 13.2</td>
<td>45.4 ± 13.5</td>
<td>29.0 ± 9.7</td>
<td>40.2 ± 12.2</td>
<td>37.4 ± 15.0</td>
<td>50.6 ± 13.1</td>
<td>$P &lt; .001^{a}$</td>
<td>$P = .448$</td>
</tr>
<tr>
<td>5-RM seated rows [kg]</td>
<td>$P = .192^{b}$</td>
<td>41.8 ± 14.7</td>
<td>57.6 ± 15.0</td>
<td>38.3 ± 13.9</td>
<td>53.9 ± 16.4</td>
<td>45.3 ± 15.1</td>
<td>61.3 ± 13.0</td>
<td>$P &lt; .001^{a}$</td>
<td>$P = .933$</td>
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<tr>
<td><strong>Body analysis</strong></td>
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<tr>
<td>BMI [kg/m²]</td>
<td>$P = .103^{b}$</td>
<td>33.7 ± 4.6</td>
<td>33.6 ± 4.6</td>
<td>35.1 ± 4.4</td>
<td>34.8 ± 4.6</td>
<td>32.4 ± 4.6</td>
<td>32.4 ± 4.5</td>
<td>$P = .588$</td>
<td>$P = .217$</td>
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<tr>
<td>Body fat [kg]</td>
<td>$P = .103^{b}$</td>
<td>39.0 ± 7.5</td>
<td>37.1 ± 8.2</td>
<td>41.2 ± 6.9</td>
<td>38.8 ± 7.7</td>
<td>36.7 ± 7.6</td>
<td>35.2 ± 8.6</td>
<td>$P = .013^{a}$</td>
<td>$P = .556$</td>
</tr>
<tr>
<td>Fat-free mass [kg]</td>
<td>$P = .578^{b}$</td>
<td>58.7 ± 12.1</td>
<td>60.4 ± 12.8</td>
<td>57.5 ± 13.1</td>
<td>59.4 ± 14.4</td>
<td>60.0 ± 11.3</td>
<td>61.6 ± 11.3</td>
<td>$P = .015^{a}$</td>
<td>$P = .812$</td>
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<tr>
<td><strong>Glucose regulation</strong></td>
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<tr>
<td>Plasma HbA1c [%]</td>
<td>$P = .780^{b}$</td>
<td>7.0 ± 0.9</td>
<td>6.7 ± 0.7</td>
<td>7.0 ± 0.8</td>
<td>6.6 ± 0.7</td>
<td>7.1 ± 1.0</td>
<td>6.8 ± 0.8</td>
<td>$P = .001^{a}$</td>
<td>$P = .516$</td>
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<tr>
<td>Serum glucose [mg/dL]</td>
<td>$P = .834^{b}$</td>
<td>131 ± 30</td>
<td>123 ± 21</td>
<td>132 ± 21</td>
<td>123 ± 19</td>
<td>130 ± 37</td>
<td>123 ± 24</td>
<td>$P = .096$</td>
<td>$P = .794$</td>
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<tr>
<td>Serum insulin [microU/mL]</td>
<td>$P = 0.01^{a,b}$</td>
<td>20.0 ± 8.9</td>
<td>17.5 ± 7.9</td>
<td>24.1 ± 10.1</td>
<td>19.7 ± 8.6</td>
<td>15.9 ± 5.4</td>
<td>15.3 ± 6.7</td>
<td>$P = .030^{ad}$</td>
<td>$P = .606^{d}$</td>
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<tr>
<td>HOMA-IR index</td>
<td>$P = 0.012^{a,b}$</td>
<td>6.4 ± 3.1</td>
<td>5.3 ± 2.4</td>
<td>7.8 ± 3.3</td>
<td>5.9 ± 2.4</td>
<td>5.1 ± 2.0</td>
<td>4.6 ± 2.1</td>
<td>$P = .029^{ad}$</td>
<td>$P = .755^{d}$</td>
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<td><strong>Lipid profile</strong></td>
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<td>Serum triglycerides [mg/dL]</td>
<td>$P = .320^{b}$</td>
<td>209 ± 89</td>
<td>178 ± 73</td>
<td>226 ± 81</td>
<td>173 ± 61</td>
<td>193 ± 96</td>
<td>182 ± 86</td>
<td>$P = .024^{a}$</td>
<td>$P = .124$</td>
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<tr>
<td>Serum total cholesterol [mg/dL]</td>
<td>$P = .732^{b}$</td>
<td>181 ± 37</td>
<td>177 ± 39</td>
<td>179 ± 40</td>
<td>173 ± 43</td>
<td>184 ± 36</td>
<td>181 ± 36</td>
<td>$P = .259$</td>
<td>$P = .742$</td>
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<tr>
<td>Serum LDL/HDL ratio</td>
<td>$P = .542^{b}$</td>
<td>2.6 ± 0.8</td>
<td>2.6 ± 0.7</td>
<td>2.5 ± 0.8</td>
<td>2.5 ± 0.8</td>
<td>2.7 ± 0.8</td>
<td>2.6 ± 0.7</td>
<td>$P = .386$</td>
<td>$P = .862$</td>
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<td><strong>Anti-/Pro-inflammatory molecule levels</strong></td>
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<tr>
<td>Serum IL-6 [pg/mL]</td>
<td>$P = .744^{c}$</td>
<td>5.2 ± 4.1</td>
<td>5.7 ± 6.6</td>
<td>5.0 ± 3.7</td>
<td>6.6 ± 9.0</td>
<td>5.4 ± 4.6</td>
<td>4.9 ± 3.0</td>
<td>$P = .691$</td>
<td>$P = .426$</td>
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<tr>
<td>Serum IL-10 [pg/mL]</td>
<td>$P = .775^{c}$</td>
<td>7.7 ± 2.6</td>
<td>7.3 ± 3.0</td>
<td>7.5 ± 1.8</td>
<td>7.4 ± 2.1</td>
<td>8.0 ± 3.2</td>
<td>7.3 ± 3.8</td>
<td>$P = .163$</td>
<td>$P = .284$</td>
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<tr>
<td>Serum TNFα [pg/mL]</td>
<td>$P = .653^{c}$</td>
<td>6.4 ± 2.4</td>
<td>6.8 ± 3.1</td>
<td>6.1 ± 2.1</td>
<td>6.8 ± 2.9</td>
<td>6.6 ± 2.7</td>
<td>6.7 ± 3.4</td>
<td>$P = .280$</td>
<td>$P = .439$</td>
</tr>
</tbody>
</table>

*Note:* Variables in bold print were defined as outcome variables prior to the study. Data are means ± SD.

Abbreviations: 5-RM, 5-repetition maximum; ANOVA, analysis of variance; BMI, body mass index; C group, control group (fed group); F group, fasting group; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR index, homeostatic model assessment of insulin resistance index (fasting glucose [mg/dL] × fasting insulin [microU/mL]/405); IL, interleukin; LDL, low-density lipoprotein; TNFα, tumor necrosis factor α.

*Significant result.

.Result from the *t* test.

.Result from the Mann-Whitney *U*-test.

.For transformed values (Box-Cox transformation).

.One missing value.
the training intervention (T2). All subjects were instructed not to change their dietary habits during the study period. Dietary records were kept for 24 hours in the first and last training week to ensure that caloric intake did not differ. The following procedures were performed at T1 and T2: venous blood was collected after a 12-h overnight fast and before medication intake in the early morning. Shortly thereafter, subjects were weighed and a bioelectric impedance analysis (BIA) was performed to determine body fat and muscle mass. One to two days later, the subjects’ endurance performance was tested on a cycle ergometer coupled to an electrocardiogram during an incremental step test. Strength tests were carried out at the beginning of the first and last training sessions.

2.2 Subjects

The subjects were recruited via newspaper advertisements (2017-2018). The inclusion criteria required the subjects to be at least 40 years of age, to have T2DM (without insulin treatment) and to be untrained. Exclusion criteria included the presence of severe infections or chronic diseases other than T2DM (e.g., HIV, hepatitis, cancer, and chronic obstructive pulmonary disease) as well as of severe cardiovascular complications. Thirty T2DM patients (19 men, 11 women) participated in the study (age: 60 ± 8 years). The duration of their disease was 6 ± 4 years. Well-controlled hypertension was observed in 24 patients. Twenty-five of the patients took antidiabetic drugs (13 in the F group and 12 in the C group), 24 took antihypertensive drugs (12 in the F group and 12 in the C group), 8 took antihyperlipidemic/antihypercholesterolemia drugs (4 in the F group and 4 in the C group), and 20 of the patients took other therapeutic agents (see Table S1).

It was determined by a questionnaire that the subjects had not exercised regularly (more than once a week) in the last year prior to the commencement of the study. The age of the patients in the two groups did not differ significantly (F: 61 ± 8 years; C: 59 ± 9 years, P = .744) nor did their disease duration (F: 6 ± 5 years; C: 6 ± 4 years, P = .683), body mass index (BMI), body composition, glycemic control (glycated hemoglobin: HbA1c) and fasting glucose values, lipid values, and (anti-)inflammatory molecule levels (Table 1). Insulin concentrations and the homeostatic model assessment for insulin resistance (HOMA-IR) index (calculated from glucose and insulin values) differed significantly between the groups.

2.3 Training intervention

The training sessions took place at a local fitness center (“Medisport Landwehr”). The training was performed three times a week on nonconsecutive days between 7 AM and 10 AM for a total of 8 weeks and supervised by professional sports coaches. All subjects completed exactly the same number of training sessions. If a patient could not take part in a training session, it was rescheduled for a later point at the end. The attendance was thus 100%. Patients from the F group were instructed to train in the fasted state after a 12-hour overnight fasting period, while patients from the C group were instructed to eat breakfast prior to the training session (1-2 hours before the start of the training session). Both groups performed the same exercises (intensity and duration). A combined endurance/strength training program was conducted in accordance with the American Diabetes Association guidelines.1 The endurance training included 30-minute cycling on a bicycle ergometer (“C842i”, Precor). The endurance practice intensity (heart rate measured by heart rate monitors (“FS2C”, Polar) was individually adapted to the heart rate corresponding to ~70%-80% of the subject's peak heart rate based on the endurance test conducted prior to the commencement of training. All patients kept up this intensity for 30 minutes during each session. The strength training included: chest presses, seated rows, abdominal crunches, back extensions, and leg presses (strength training equipment made by Nautilus). The patients performed three sets of 15 repetitions with approximately 65% of the 1-repetition maximum (RM). The training weight was increased if the subjects were able to perform a greater number of repetitions than initially instructed. There were no adverse events during the training period.

2.4 BIA

Body fat and muscle mass were determined by the “BIA 101 Akern” and the “Insunded Akern” software (Akern). The system shows high accuracy.16 Patients were instructed to empty their bladder before the measurements. Analyses were performed in the lying position after 10 minutes of rest.

2.5 Physical performance tests

An endurance test was performed on an upright bike coupled with an ECG (“CardioPro-System”, Zimmer Medizintechnik). Subjects were tested with the following stopping criteria: muscular exhaustion, angina pectoris, ischemia, paleness, cyanosis, arrhythmia, respiratory insufficiency, hypertension (systolic blood pressure > 250 mm Hg or diastolic blood pressure > 115 mm Hg), aberration, dizziness, and/or coordination problems. Starting at 25 W resistance, the intensity gradually increased by 25 W every 2 minutes. The subjects were always tested at the same time of day, and they were instructed not to engage in physically exhausting activities 24 hours before the measurement. Strength tests were performed as 5-RM tests as described previously.17
2.6 | Measurement of clinical blood variables and interleukin (IL)-6, IL-10 and tumor necrosis factor (TNF)α serum levels

Clinical blood variables (HbA1c, glucose, insulin, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL)) were analyzed using standard methods. The HOMA-IR index was calculated from the glucose and insulin values. Enzyme-linked immunosorbent assay (ELISA) kits from R&D systems were used to quantify the serum levels of molecules in the blood: IL-6 (article number: D6050), IL-10 (D1000B), and TNFα (DTA00D). The lower detection limits were 0.7, 3.9, and 4.0 pg/mL, respectively. If the molecules in a sample were below the detection limit, the value for the sample was set to the value corresponding to the detection limit. All measurements were performed in duplicate.

2.7 | Statistical analyses

Statistical analyses were carried out using the “SPSS” program (v. 24.0, SPSS Incorporation). Normal distribution was tested using the Shapiro-Wilk test. The following data were not normally distributed in at least one group: age at T1, duration of the disease at T1, IL-6 at T1 and T2, IL-10 at T1 and T2, TNFα at T1 and T2. Differences between the groups (F and C) at baseline (T1) were tested using the independent t test for normally distributed data (Student’s t test if Levene’s test showed homogenous variances or Welch’s t test if it did not show homogenous variances) and the Mann-Whitney U-test for non-normally distributed data. The analysis of variance (ANOVA) was performed to detect time and time × group (fasted state/fed state) effects, irrespective of (non-)normality of data due to robustness of mixed ANOVA against violation of the normal distribution assumption. Levene’s test indicated homogeneity of the error variances for all variables except for “insulin” and “HOMA-IR index”. The insulin and HOMA-IR index values were thus transformed by Box-Cox transformation prior to ANOVA. The level of significance was set at $P \leq .05$.

3 | RESULTS

The participants’ physical fitness data as well as their BMI, body composition, glycemic control, lipid profile, and (anti-)inflammatory state data are presented in Table 1. Independently from exercising either in the fasted or in the fed state, physical training resulted in improvements of the subjects’ physical fitness, body composition, glucose regulation (HbA1c values, insulin values, HOMA-IR index), and fasting triglyceride values. The training had no effect on BMI, serum fasting glucose, total cholesterol or LDL/HDL ratio. IL-6, IL-10, and TNFα levels were also not affected by the training. ANOVA did not reveal any group-specific effects.

4 | DISCUSSION

The 8-week combined endurance/strength training performed in this study significantly affected the health-related variables in T2DM patients, irrespective of their nutritional state (fasted versus fed) during the exercise sessions.

However, some studies involving healthy subjects indicate that long-term training in the fasted state is associated with up-regulation of components of the oxidative metabolism as well as with improvements in glucose regulation. In line with these findings, proteins for muscle fat transport and oxidation (muscle fatty acid translocase CD36 protein, fatty acid binding protein or citrate synthase) were more extensively up-regulated following training in the fasted state.

Furthermore, higher increases in the muscle glucose transporter (GLUT)-4 protein contents were observed. Although there is no direct evidence that training in the fasted state results in higher body fat loss, Van Proeyen et al demonstrated that training in the fasted state prevents adipose tissue mass gains in lean individuals with a high-fat diet, whereas training in the fed state did not. Increases/Decreases in fat mass are relevant for glucose regulation as fat mass coincides with the release of mediators of inflammation from adipose tissue macrophages, for example TNFα, which directly and indirectly affects glucose homeostasis by its action on different cell types. With reference to these studies, it has been speculated that the effects of training in the fasted state could be highly beneficial and more pronounced in T2DM patients under pathological conditions than in healthy individuals. This assumption was, however, not confirmed by the present study data.

While the F and C group participants did not differ significantly in any variable that was defined as an outcome variable in the study during the registration of the trial (BMI, HbA1c, fasting glucose, triglycerides, total cholesterol, LDL/HDL, serum IL-6, IL-10, and TNFα), the insulin concentration and HOMA-IR index (indirect measure of insulin resistance and calculated from fasting glucose and insulin values) differed between the groups. Unfortunately, insulin levels and HOMA-IR index were determined a posteriori (after completion of the study). These are clear limitations of the study and must be taken into consideration when interpreting the study results. In this regard, it should, however, be noted that there can be a relatively high intra-individual (day-to-day) variability in fasting insulin values (as well as glucose values) and HOMA index. Future studies should use better measures to quantify insulin sensitivity, for example, the hyperinsulinemic-euglycemic clamp method as the “gold standard” for determining whole-body insulin sensitivity.
One explanation of why training in the fasted state had no superior effects in the present study could be related to the fact that T2DM at an early stage is often accompanied by hyperinsulinemia. Some previous studies indicate that there are lower blood insulin levels, elevated blood free fatty acid concentrations as well as increased fat oxidation rates in healthy subjects during acute exercise in the fasted state. This led to the assumption that repeated exercise in the fasted state could be instrumental in improving oxidative metabolism and glycemic control (lipotoxicity-induced insulin resistance) in the long-term. At this point, it can be speculated that insulin levels during exercise in the fasted state are lower in healthy individuals than in the T2DM patients included in the present study (some of the patients had hyperinsulinemia), and that—due to insulin's anabolic effects—catabolic processes could have been affected. Further studies comparing adaptations to acute and chronic exercise in the fasted state in solely hyperinsulinemic versus normoinsulinemic patients are necessary to clarify this circumstance. In this context, it would also be interesting to evaluate effects of training in the fasted state in type 2 diabetes patients who receive insulin treatment (when insulin production becomes insufficient at a later stage of the disease).

In further studies, it would also be useful to measure fat oxidation during exercise to determine whether fat oxidation also increases in patients with T2DM during exercise in the fasted state. Spirometric measurements including the recording of the respiratory exchange ratio pre- and post-intervention could help estimate long-term metabolic adaptations and changes in oxidative metabolism.

Discrepancies in the results of the studies could also be explained by variations in exercise protocols. In the present study, a combined endurance/strength training program was conducted which conformed to the recommendations of the American Diabetes Association thus representing an optimal training regime for this particular patient group. In most of the aforementioned studies, endurance training was performed. The question is whether different adaptations can result from endurance or strength training or their combination. It must also be answered whether combining endurance and strength exercise in the fasted state in the same training session are most suitable for inducing health adaptations. The strength training used in the present study was a strength endurance training during which the patients performed a relatively high number of repetitions. In any case, both types of exercise (endurance and strength) can lead to a fall in cellular energy in the muscle and a subsequent activation of the AMP-activated protein kinase (AMPK). AMPK activation has, in turn, been considered to play an important role in stimulating fatty acid oxidation and glucose uptake (GLUT-4 translocation) during exercise. Furthermore, AMPK activation could be important for stimulating mitochondrial biogenesis as well as GLUT-4 expression (long-term adaptations). However, studies regarding the role of AMPK in skeletal muscle are partly controversial. There are indices from animal studies and a human study involving healthy subjects that exercise and fasting can have synergistic effects for the AMPK activation. Contrary, in another human study involving healthy subjects, increases in AMPK phosphorylation following acute exercise were not further amplified by fasting. Thus, molecular mechanisms triggering effects of different exercise programs combined with food withdrawal have to be further explored to better understand cause and effect. To do so, other training modalities than those used in the present study (type: endurance and/or strength, other frequencies/intensities) should be considered.

Medication intake could also affect cellular and molecular mechanisms and study outcomes. Due to medical and ethical reasons, patients continued taking their medication throughout the study period. The dosage was not changed during the course of the study to minimize bias. Nevertheless, it has been demonstrated that antidiabetic medication can influence training-induced adaptations. Metformin, for example, can attenuate some of the effects of exercise training on cardiovascular risk factors. However, medication intake among subjects from both groups was quite similar in the present study. This reduces the possible influence of medication intake on group-specific effects.

Finally, the relatively low number of subjects in this preliminary study must be considered when interpreting the results.

4.1 Perspectives

Regular exercise is highly recommended in the therapy of T2DM. Training in the overnight-fasted state has received considerable attention. However, the preliminary study’s data do not provide any evidence that the nutritional state (overnight-fasted or fed) in regular physical training plays a significant role for training-induced adaptations in T2DM patients. Full trials (using other training protocols as well) should be carried out to arrive at a more comprehensive understanding of the relevance of exercising before breakfast for training-induced adaptations in T2DM patients.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.
REFERENCES


SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.